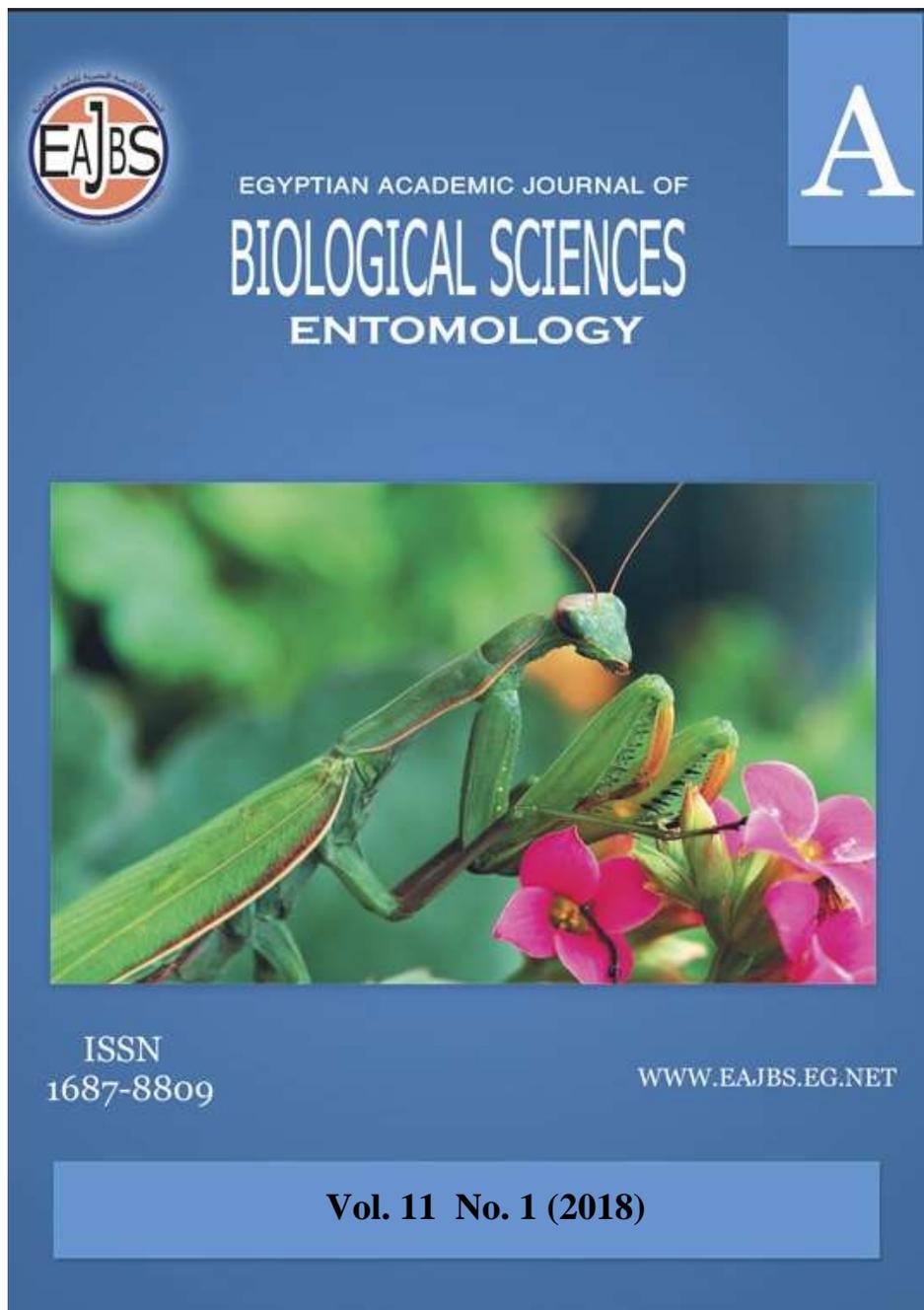


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University. Entomology Journal publishes original research papers and reviews from any entomological discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry, development, genetics, systematics, morphology, evolution, control of insects, arachnids, and general entomology.
www.eajbs.eg.net



Direct Effects of Temperature Changes on Biochemical and Enzymatic for Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

Aida, S.Kamel¹, Walaa, E. Gamil² and Dahi, H. F. ²

1 - Entomology Department, Faculty of Science, Benha University, Egypt.

2 - Plant Protection Research Institute, Agricultural Research Centre, Giza, Egypt.

Email: aida_momtaz@hotmail.com

ARTICLE INFO

Article History

Received: 18/1/2018

Accepted: 20/2/2018

Keywords:

Temperature, *Spodoptera littoralis*, biochemical analyses, metabolic, digestive, neural and detoxification enzymes.

ABSTRACT

It is important to understand the effects of increasing temperatures due to global warming on Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) which damages a wide variety of crops in the Middle East and is one of the major economic pests of cotton in Egypt. Therefore, we are investigating five different constant temperatures at 15, 20, 25, 30 and 35°C as different thermal effects on biochemical parameters and enzymes of fourth larval instars. Biochemical analyses were performed to determine the effects of these different constant temperatures on the amount of total proteins, lipids, carbohydrates and free amino acids as well as metabolic, digestive, neural and detoxification enzymes. It's clear from the results that the total amount of proteins, free amino acids as well as, the activity levels of proteases were increased significantly by raising of temperatures to reach their maximum activities at 30°C. While the total amount of lipids, Carbohydrates were decreased significantly by raising of temperatures. Moreover, activity levels of amylase, invertase and Trehalase were decreased at 15°C. Amylase and invertase reached their maximum activity at 30°C, while Trehalase reached its maximum activity at 20°C. There was severe reduction in carbohydrate enzymes activities at 35°C. On other hands, the activity levels of total lipids and lipase were increased significantly at lower temperatures 15°C and 20°C, no significant changes were detected between other temperatures. The activity levels of GOT and GPT increased significantly and reached their maximum level at 20°C. However, GOT and GPT at other temperature were significantly different. The level of GST Enzyme was increased significantly at 30°C, while, its activity level was decreased at 15°C. Moreover, AchE, Alpha (EST- α) and beta esterase (EST- β) enzymes were increased gradually at 15, 20 and 25 °C. They reached their maximum activities at 25°C followed by the reduction in activity levels at 30°C and 35°C.

INTRODUCTION

Insects face adverse stressors on a daily basis, for instance, fluctuations in temperatures and thermal stresses which may effect on their fitness and metabolism (Rinehart *et al.*, 2000). Temperature is profound biotic environmental factors that induce physiological changes in insects resulting in rapid metabolic variation, which can lead to disorder affecting on their lives (Parmesan&Yohe 2003). On other hands, some studies on the effects of heat on insect metabolism demonstrated some adaptability to thermally challenging environments (Neven, 2000).

Insects are relatively easily affected by thermal stress, which can quickly elevate their body temperatures to lethal levels (Denlinger & Yocum, 1998). Many authors concluded that, as a result of global warming, the frequency and degree of appearance of high temperatures are predicted to increase substantially (Easterling *et al.*, 2000; Diffenbaugh *et al.*, 2005). In addition, Parmesan (2006) noted that the change of climate may cause insect species to spread at the mean rate of 6.1 km per decade. It can influence insect pests and the size of damage caused by them: directly through their development, reproduction and distribution and indirectly by altering host physiology and defence mechanisms. Moreover, temperatures tend to enhance insect survival because it accelerated metabolism which may lead to higher consumption, growth, and development rates. Faster development, in turn, may lead to population increases via reduced generation time and decreased warmer late winter and early-spring (Bale *et al.*, 2002). Metabolism, growth and reproduction increase exponentially with warming. Global warming is producing increases in both average temperatures and in the frequency and severity of heat waves (Stone *et al.*, 2010). Moreover, because insects can be killed by short exposure to an extremely high temperature, heat treatments can be applied to control horticultural and stored-product pests, with few insecticidal applications, decreasing the environmental threat, (Cui *et al.*, 2008 and Hansen *et al.*, 2011). Recent studies by many authors suggested that the conditions in the upper elevation and also in higher latitude would become more suitable for organisms, because global warming will exceed threshold of tolerance, in almost all insect species, which, in turn, accelerates cellular energetic demands, (Englund *et al.*, 2011; Lemoine & Burkepile, 2012). Insect metabolic rates are highly sensitive to temperature, roughly doubling with an increase of 10°C across the full range of regularly experienced temperatures (Berggren *et al.*, 2009). Temperature affects diffusion, membrane fluidity, nucleic acid stability, salt and gas solubility, and, significantly, the behavior of enzymes (Lee *et al.*, 2007).

The Egyptian cotton leafworm, *Spodoptera littoralis* is distributed throughout the world. It is a serious or major pest of cultivated crops primarily in tropical and subtropical regions, in Africa, Southern Europe, Middle East and Asia (Pineda *et al.*, 2004). *S. littoralis* is one of the major economic pests of cotton in Egypt that causes considerable damage to many other vegetables and crops (Dahi *et al.*, 2009). *S. littoralis* attacks all major crops in Egypt, including cotton, clover, corn, cabbage, cowpea, castor bean, sweet potato, lettuce, tomato, pepper, okra, mulberry, soybeans etc. (El-Aswad *et al.*, 2003). Enzymes are proteins that are critical to catalyzing reactions (Brooker *et al.*, 2011). One of the properties of enzymes that makes them as important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze (Kumar *et al.*, 2005). Previous studies by (Hochachka & Somero, 2002) found that temperature changes affected on the binding of a substrate to the enzyme, causing a shift in the Michaelis

constant (Km), thereby affecting immediate metabolic compensation. Moreover, An and Choi (2010), suggested that thermal stress may decrease the antioxidant state and cause oxidative stress.

The aim of this work is trying to understand the effects of increasing temperature due to global warming on *S. littoralis*. Using different temperatures in order to examine their effects on the biochemical parameters such as total proteins, lipids, carbohydrates and free amino acids as well as the activity levels of enzymes groups (metabolic, digestive, neural and detoxification enzymes) of fourth larval instars of *S. littoralis*.

MATERIALS AND METHODS

Maintenance of *Spodoptera littoralis* Culture:-

The original colony of the cotton leaf worm *S.littoralis* was obtained from a well-established culture at the Department of Cotton Leaf worm; Plant Protection Research Institute. The insects were maintained under constant conditions of 15, 20, 25, 30 and 35± 2⁰ C, 70 ± 5% R.H. and 12:12 (L: D) photoperiod, separately. Larvae were reared on fresh castor oil leaves, (*Ricinus communis* L.) supplied daily in sufficient amounts. Maintenance of the different developmental stages was conducted according to method described by Gamil, (2004).

Biochemical Bioassay:

1. Sample Preparation:

Twenty-five of 4th larval instars of *S. littoralis* from each rearing temperature degrees were collected in a chilled glass. These larvae were then homogenized in phosphate buffer (PH.7) using a Teflon tissue homogenizer. After homogenation, supernatants were kept in a deep freezer at -20⁰C till use for biochemical assays. The insects were homogenized in distilled water (50 mg. /1 ml.) The homogenates were centrifuged at 8000 rpm for 15 min at 5⁰C in refrigerated centrifuge. The deposits were discarded and the supernatant was kept in a deep freezer till used for the determination of the following:

2. Main Contents:

- Total soluble protein as described by Bradford (1976).
- Total carbohydrates according to Singh and Sinha (1977).
- Total lipids according to Knight *et al.* (1972).
- Free Amino acid assayed by ninhydrin reagent according to Lee and Takabashi (1966).

3. Enzymes Assay:

The Following Enzymes Activities Were Determined:

- Proteases activity was measured as described by Tatchell *et al.*, (1972).
- Carbohydrates hydrolyzing enzymes; Amylase, Trehalase and Invertase were determined by the method of Ishaaya and Swiriski (1976), using starch, trehalose and sucrose as substrates.
- Lipase activity was measured as described by Tsujita *et al.* (1989).
- Acetyl choline-esterase activity was determined using acetylcholine bromide (AChBr) as substrate according to the method described by Simpson *et al.* (1964).
- Non-specific α and β esterase activities were measured as described by Asperen, (1962) using α naphthyl acetate and β naphthyl acetate, respectively, as substrates.
- Glutathione S-transferase activity (GST) was determined spectrophotometrically at 340 nm according to the method of Habig *et al.* (1974).
- Acid and alkaline phosphatase activities were measured from the larval haemolymph as described by Laufer and Schin (1971).

Statistical Analysis:

Data from all experiments were subjected to analysis of variance (ANOVA) Using the computer program SAS (Statistical Analysis Systems).

RESULTS AND DISCUSSION

Several studies done on various insect species have shown that temperature rise leads to increased metabolic rate, thus decreasing the development period and causing an increase in stored foods (Lale *et al.*, 2003; Taveras *et al.*, 2004; Khriut *et al.*, 2006; Coracini *et al.*, 2007). In addition, a number of physiological stress responses occur in insects as a result of variations in temperature. One reaction to thermal stress is the generation of reactive oxygen species (ROS), which can be harmful by causing oxidative damage. (Ali *et al.*, 2017).

Biochemical Analysis:

Temperature has profound effects on chemical and biochemical reactions (Hochachka and Somero, 2002). Moreover, temperature increases, the higher kinetic energy of biochemical reactions speed up the rate of metabolic processes, scaling up to affect the physiology and behaviour of individual organisms (Angilletta, 2009). Protein has always been interesting biochemical tool for insect biochemists because of their potential role in growth, development, morphogenesis and many intermediaries of the metabolic pathway of insects Kar *et al.*, (1994). In the current study as seen in (Table.1&Fig.1), it is clear that the levels of total proteins recorded, a gradual increase in haemolymph during the development of fourth larval instars by raising temperature at 15, 20, 25 and 30°C. They were (47.67, 57.67, 62.33 and 59.33 mg/g.b.wt. respectively, but there were no significant differences between the levels of total protein in the last three degrees of temperature. Meanwhile, at 35°C the amount of total protein (22.67 mg/g.b.wt) decreased significantly in comparison with total protein levels at previous degrees of temperature. In addition, free amino acid increased gradually by increasing of temperatures at 15, 20, 25 and 30 °C, they were 376.33, 627.0, 732.3 and 776.3 U_g alanine/ml. respectively. There were no significant changes between amounts of free amino acids at 20 and 30°C, while, they decreased significantly at 35°C it was 73.33 U_g alanine/ml. These results may be revealed to Caterpillar growth rates increase with raising the temperature and the larvae consumed more protein rich diets. Our results agree with the finding of (Kingsolver, 2006 and Lee and Roh 2010). In the previous study, Nagata and Kobayashi (1990) had reported an increase in protein synthesis during feeding stage in *bombyx mori* in haemolymph and increase the soluble protein level during pupal development could be attributed to compensatory replacement of protein that utilized for formation of pupation. Moreover, Martin *et al.*, (1969) reported that the increase of protein levels in haemolymph during oncogenic development of fifth larval instars of silkworm *bombyx mori*. The increase of protein levels of haemolymph due to the synthesis of new proteins by tissue and release into haemolymph. Hachiya *et al.*, (2007) reported that proteins denature more rapidly at higher temperatures, which, in turn, requires greater rates of protein synthesis and repair to maintain basic cellular function. On contrast Malik and Malik (2009) were found when the larvae and pupae exposed to selected higher temperatures (31 °C and 36°C), a significant decrease in the protein levels of haemolymph was noticed and the order of decrease was to be more at 36°C than at 31°C. Relatively higher increase in the free amino acid levels in the haemolymph presumably provides protective cover to tissues against high temperature by an increase in osmolality and reduction in evaporative

water loss. On the other hand Kiranet *et al.*, (1998) reported that the fat body synthesizes a number of protein and release them into the haemolymph during active larval period. This clearance was confirmed by Willmer *et al.*, (2004) they reported that high temperature affected all biological process including the structure of proteins and biological members and rates of biochemical and physiological reactions. Moreover, (Gullan and Cranston, 2005) concluded the high temperature tended to kill insect's cells by denaturing proteins, altering membrane, enzyme structures and properties, by the loss of water (dehydration) and they offer a rich potential for pest management strategies. While the previous study carried by Neven (2000) on the codling moth were reared at different temperature 10,15, 20, 25 and 30°C, the moth subjected to acute heat treatments had a maximal carbon dioxide evolution rate, respiration increase in response to increasing temperatures to critical upper limit. After this point, respiration decreased. So, death occurs soon after the respiration rate drop even if the insect returned to normal thermal condition indicating that death cells, death is occurring and denatured protein.

In contrast, the recent study carried out by Lemoine and Shantz (2016) on *Spodoptera exigua* cleared the decreasing of protein at 30°C due to, nitrogen digestibility declined by almost 25% at 30°C compared with 25 °C. Reduced nitrogen digestibility, coupled with increased cellular division and somatic growth rates, may be responsible for the observed strengthening of protein limitation at high temperatures.

Lepidopteran larvae may be particularly vulnerable to environmental warming because their rapid growth rates demand high protein foods (Lee *et al.*, 2004) Ehsan *et al.*, (2011) were revealed the fourth larval instars of *Pistachio* white leaf borer reared at 25°C had a lower level of glycogen and higher level of protein in comparison with those larvae reared at 35°C. Glycogen is storage form of energy, therefore temperature rise leads to increase metabolic rate, decrease the development period and cause an increase in stored foods. Protein content in larvae reared at 25°C was significantly higher than those reared at 35°C, but glycogen content in the larvae reared at 35°C was more than larvae reared at 25°C.

In our study the amount of total lipids as seen in (Table.1& Fig.1) were recorded an increasing significantly in haemolymph at lower temperature 15°C and 20°C. They were 14.98 and 10.33mg/g.b.wt. However, the recorded decrease in total lipid levels by gradual increase of temperature at 25,30 and 35°C they were 6.7, 6.80 and 7.03mg/g.b.wt., respectively. No significant changes in the total lipid levels recorded between these degrees of temperatures. These results may be due to increasing in food consumption at lower degrees of temperatures to provide energy. These results agree with findings of. Hochachka and Somero, (1973), they revealed the increase in the contents of total lipids, free fatty acids and phospholipids to increase in the food consumption. The increase in free fatty acids may provide the lipoprotein enzymes, with an environment to modulate the latter's activity at low temperature. On the other hand, many authors (Ellis *et al.*, 2002; Costamagna and Landis, 2004) reported that the orders Lepidoptera and orthoptera use lipids and stored carbohydrates as the main energy source. Lee and Roh (2010) concluded that lipids storage efficiency was lower in larvae of *Spodoptera exigua* at 18°C than at 26 °C, and was similar to those at 34 °C. Dooremalen *et al.*, (2011) found that in *Philosamia ricini*, lipid composition may be an important trait under lying fitness response to temperature, because it affects membrane fluidity as well as a viability of stored energy reserves. The observed increase in the phospholipid content, larvae showed greatly increased activity of those mitochondrial enzymes

which were membrane associated, and thus the former retained their reticular structures intact during cold exposure. Therefore, *Philosamia ricini* larvae were exposed to higher temperature of 35°C, monovalent cations like Na⁺ and K⁺ increased whereas divalent cations like Ca²⁺ and Mg²⁺ decreased, the percent changes observed being more at 36°C than at highest. At 31°C. The haemolymph monovalent cations were hyper feeding larvae and divalent cations were hypo-regulated at the quantity of silk synthesized by their silk glands.

In current study, the amount of total carbohydrates as seen in (Table.1. & Fig.1) increased significantly at 15°C and 20°C they were 20.43 and 26.19mg/g.b.wt. There was significant difference in the total carbohydrates between these temperatures while decreased at 25°C, 30°C and 35°C they were 9.63, 9.40 and 4.77mg/g.b.wt. There were no significant changes between 25°C and 30°C while the amount of total carbohydrates decrease significantly at 35°C. These results disagree with Sonmez and Gulel (2008), they concluded that a low temperature decreases the total carbohydrates and protein amounts of the pest *Acanthoscelides obtectus*. They recommended that Storage should be kept at 10-15°C so that, *A.obtectus* and other possible pests give minimal damage crops.

Table (1): Effect of different constant temperatures on biochemical of *S. littoralis*

Temp. (°C)	Total proteins mg/g.b.wt Mean ± SE	Total lipids mg/g.b.wt Mean ± SE	Total carbohydrates mg/g.b.wt Mean ± SE	Free amino acids Ug alanine/ml Mean ± SE
15	47.67 ± 1.20 ^b	14.98 ± 0.77 ^a	20.43 ± 0.82 ^b	376.3 ± 9.93 ^c
20	57.67 ± 1.45 ^a	10.33 ± 0.48 ^b	26.19 ± 0.85 ^a	627.0 ± 11.44 ^b
25	62.33 ± 1.76 ^a	06.72 ± 0.17 ^c	09.63 ± 0.57 ^c	732.3 ± 13.42 ^a
30	59.33 ± 1.20 ^a	06.80 ± 0.12 ^c	09.40 ± 0.59 ^c	776.3 ± 10.17 ^a
35	22.67 ± 1.45 ^c	07.03 ± 0.26 ^c	04.77 ± 0.64 ^d	73.30 ± 6.69 ^d

Note: Means with the same letter in the same row are not significantly different

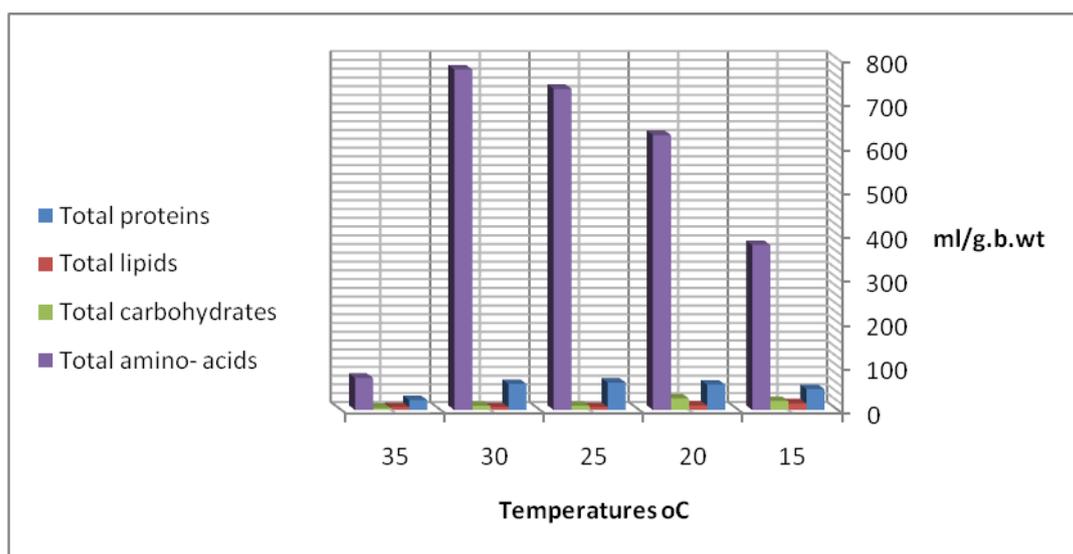


Fig (1): Effect of different constant temperatures on biochemical of 4th *S. littoralis* larval instars

Enzyme Assay:

The optimum temperature for enzyme reactions is generally defined as the temperature at which the maximum reaction rate is achieved Takaya (2005). The fact that each enzyme behaves differently under various temperature regimes highlights the fact that each enzyme has an optimum temperature activity. Carbohydrates, proteases, and lipases are the three main enzymes involved in the digestion of food by insects (Callaghan *et al.*, 2002).

In our study, it is clear in (Table 2 & Fig.2) the activities of the carbohydrate hydrolyzing enzymes amylase, and invertase levels were increased by the gradual increase of temperatures at 15, 20, 25°C. They were 81.0, 117.0, 268.66 Ug glucose/min/g.b.wt., for amylase and 4079.3, 5943.3, 8502.7 Ug glucose/min/g.b.wt., for invertase. They reached their maximum activities at 30°C, there were 341.0 and 9012.0 Ug glucose/min/g.b.wt., in both amylase and invertase enzymes respectively. Their levels were decreased at 35°C which were 67.66 and 1580.0 Ug glucose/min/g.b.wt., respectively. There were significance differences between levels of amylase at all degrees of Temperature. Meanwhile there were significant differences in levels of invertase enzyme in all degrees of temperature except 25 and 30°C. In contrast, Sultan *et al.*, (2009) showed the effects of temperature on amylase were presented in salivary gland and mid gut at optimum temperature 35-40°C. Meanwhile, in our study the activity levels of Trehalase increased at 20°C, they were 1800.0 mg/g.b.wt. While activity levels decreased at 15, 25, 30 and 35°C, there were 573.33, 1370.0, 1305.0 and 1016.67 mg/g.b.wt., respectively. Proteases, which are also known as endopeptidases, enroll an important function in protein digestion. These enzymes begin the protein digestion process by breaking internal bonds in proteins. The amino acid residues vary along the peptide chain, therefore, different kind of proteinases are necessary to hydrolyze them. Based on active site group and their corresponding mechanism, digestive proteinases can be classified as serine, cysteine, and aspartic proteases (Terra and Ferreira, 2012).

In the current study in (Table 2 & Fig.2) the activity levels of Protease Enzymes were increased by increasing temperature. They, were 58.33, 85.33, 117.0 and 207.66 ug alanine/min/g.b.wt at 15, 20, 25 and 30°C respectively. There was significant reduction in enzyme level at 35°C it was 23.33 ug alanine/min/g.b.wt Pant & Gupta (1979) noted that a high proteolytic activity in second larval instar of *Philosamia. Ricini* declined steadily till late fifth instar development. The decrease was attributed to the composition of food ingested. The low proteolytic activity was due to the host plants which are rich sources of amino acids and which are consumed in large quantities by this insect. The two major proteases classes in the digestive systems of phytophagous insects are the serine and cysteine proteases (Haq *et al.*, 2004). Moreover, Srinivasan *et al.*, (2008) had reported on the midgut enzymes of various pests belonging to Lepidoptera. Serine proteases are known to dominate the larval gut environment and contribute to about 95 % of the total digestive activity in Lepidoptera.

Lipases (triacylglycerol-acyl-hydrolase EC 3.1.1.3), which catalyzes the hydrolysis of fatty acid ester bonds, are widely distributed among animals, plants and microorganisms (Naumff, 2001). In current study as seen in (Table 2 & Fig.2), the activity levels of lipase in mid gut of larvae increased significantly at lower temperatures 15 and 20°C, they were 1306.33 and 1131.0 Ug oleic acid/g.b.wt respectively. There were no significant changes between them. On the other hand, their activity levels decreased significantly when temperature raised at 25, 30 and 35°C. They were 820.33, 810.0 and 789.0 Ug oleic acid/g.b.wt. respectively. There

was no significant change between them. These results agree with finding of some previous authors Ishaaya *et al.*, (1971) concluded that enzymatic activity increased when the temperature rose from 10 to 32°C. At 10°C (i.e. below the threshold of larval development), both proteolytic and amylolytic activities in the midgut wall were less than 10 percent of that obtained at 32°C.

Detoxification enzymes in insects are generally demonstrated as the enzymatic defense against foreign compounds and play a significant role in maintaining their normal physiological functions (Mukanganyama *et al.*, 2010). Several defensive mechanisms and biochemical reactions are involved in the detoxification processes against any temperature Stress. These mechanisms predominantly involve either metabolic detoxification of the Temperature stress before it reaches to their damage, or the sensitivity changes of the larvae. The most common metabolic resistance mechanisms involve esterases, glutathione S-transferases (GSTs). Generally speaking, increase of activity of detoxification enzymes is the most universal resistant mechanism in insects. An elevation in the activity of such enzymes but surprisingly, this assumption couldn't be achieved as GST relatively decreased exposed to different constant temperature. GSTs also play an important role in stress physiology, and have been implicated in intracellular transport and various biosynthetic pathways (Wilce and Parker, 1994).

Table (2): The activity levels of digestive enzymes of 4th larval instars *S. littoralis* at different constant temperatures

Temp.°C	Protease Ug alanine/min/g.b.wt Mean ± S.E	Lipase Ug oleic acid/g.b.wt Mean ± S.E	Trehalase Ug glucose/min/g.b.wt Mean ± S.E	Invertase Ug glucose/min/g.b.wt Mean ± S.E	Amylase Ug glucose/min/g.b.wt Mean ± S.E
15	58.3 ± 2.84 ^d	1306.3 ± 48.72 ^a	573.3 ± 6.69 ^d	4079.3 ± 74.63 ^c	81.0 ± 2.080 ^d
20	85.33 ± 2.40 ^c	1131 ± 32.00 ^a	1800.0 ± 36.35 ^a	5943.3 ± 174.25 ^b	117.0 ± 6.08 ^c
25	117.00 ± 6.02 ^b	820.3 ± 2.66 ^b	1370.0 ± 62.05 ^b	8502.7 ± 175.88 ^a	268.6 ± 11.05 ^b
30	207.70 ± 7.21 ^a	810.3 ± 4.91 ^b	1305.0 ± 39.88 ^b	9012.0 ± 166.21 ^a	341.0 ± 11.50 ^a
35	23.30 ± 2.21 ^c	798.3 ± 4.78 ^b	1016.7 ± 44.09 ^c	1580.0 ± 41.47 ^d	67.7 ± 3.38 ^d

Note: Means with the same letter in the same row are not significantly different

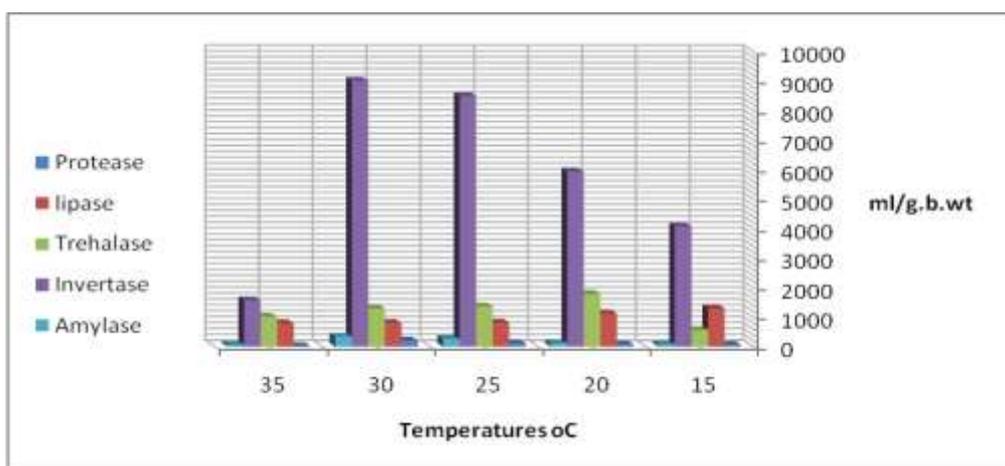


Fig (2): The activity levels of digestive enzymes of 4th larval instars *S. littoralis* at different constant temperatures.

In our study, it is clear in (Table.3. & Fig.3) the level of glutathione S-transferases (GSTs)enzyme increase significantly at 30°C. It reached its maximum activity which was 131.66 m mole sub conjugated/min/g.b.wt, while its activity level decreased at 15and 35°C, there were 27.0 and 16.0 mole sub conjugated/min /g.b.wt. respectively. There was no significant difference between them. Whereas, there were significant differences between20 and25°C.

In contrast, Zhang *et al.*,(2016) investigated the activity levels of four antioxidant enzymes,including superoxidisedismutase (SOD), catalase (CAT),glutathione-S-transferase (GSTs), and peroxidase (POD) under heat stress (0, 4, 12, 16, 20, 24 and 28°C) for 4 and 12 hours, respectively. They found that the activity of GSTs decreased obviously when the treatment temperature exceeded 24°C.This result disagree with our results seen in (Table.3). Changes in physiology of the nervous system and metabolism can be detected through the activity of acetylcholinesterase (AChE), alpha esterase (EST-α) and beta esterase (EST-β)(Débora *et al.*, 2016).Activity levels of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzymes as seen in (Table.3& Fig.3) increased significantly at 20°Cand reached their maximum levels 961.0 and 714.0 (Ux10³/g. b. wt.), respectively. They decreased at 35°C and reached the minimum levels 128.7 and 110.0(Ux10³/g. b. wt.), respectively. While activity levels of (GOT) and (GPT) at 15, 25 and 30°C were significantly difference.

Table (3): The activity levels of metabolic enzymes of 4th larval instars *S. littoralis* at different constant temperature.

Temp. °C	GST (M mole sub conjugated/min/g.b.wt) Mean ± S.E	GOT -ALT (Ux10 ³ /g.b.wt) Mean ± S.E	GPT-AST (Ux10 ³ /g.b.wt) Mean ± S.E
15	27.0 ± 01.52 ^d	457.7 ± 11.78 ^c	577.7 ± 11.78 ^b
20	102.3 ± 02.84 ^b	961.0 ± 22.23 ^a	714.0 ± 06.50 ^a
25	60.0 ± 02.30 ^c	497.3 ± 09.33 ^{ac}	296.3 ± 08.56 ^c
30	131.7 ± 10.17 ^a	557.3 ± 20.82 ^a	218.0 ± 09.29 ^d
35	16.0 ± 01.52 ^d	128.7 ± 04.48 ^d	110.0 ± 04.04 ^e

Note: Means with the same letter in the same row are not significantly different

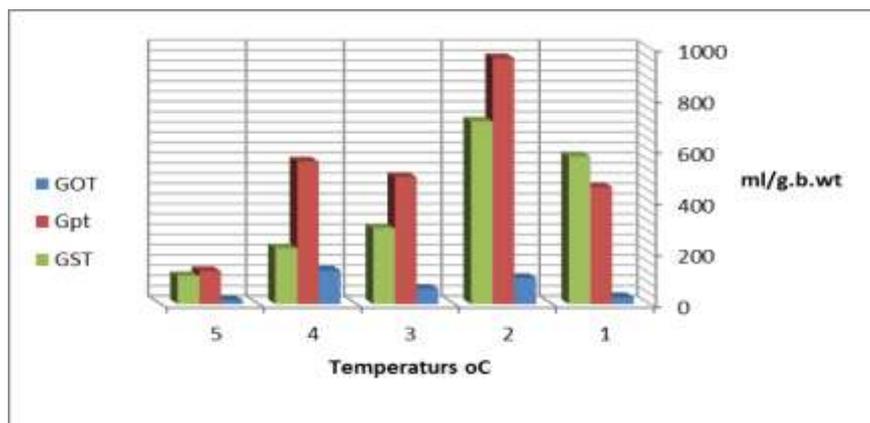


Fig (3): The activity levels of metabolic enzymes of 4th *S. littoralis* larval instars at different constant temperatures.

IN current study, as cleared in (Table.4& Fig.4). AchE enzyme activity increased gradually by increasing temperatures at 15, 20 and 25 °C they were 47.33, 67.0 and 242.0 Ug Ach Br/min/g.b.wt. respectively. While, its activity level decreased at 30°C and reached 216.0 Ug Ach Br/min/g.b.wt. Meanwhile its activity levels significantly decreased at 35°C and reached 33.7 Ug Ach Br/min/g.b.wt. Whereas, Alpha (EST- α) and beta esterase (EST- β) enzyme increased gradually at 15, 20 and 25°C they were (3036.67, 6566.6, 76985.67 ug α -naphthol/min/g.b.wt.) and (3572.0, 5603.0, 4774.33 ug β -naphthol/min/g.b.wt.) respectively, they reached its maximum activity at 25°C. Enzymes activity levels of alpha and beta decreased at 30°C, they were 4509.67 Ug α -naphthol/min/g.b.wt. and 4319.33 Ug β -naphthol/min/g.b.wt. There were sudden reduction of the enzyme activity levels at 35°C, and they were 2987.33 Ug α -naphthol/min/g.b.wt. and 1726.6 Ug β -naphthol/min/g.b.wt.

Table (4): The activity levels of acetylcholinesterase (AChE), alpha esterase (EST- α), and beta esterase (EST- β) of 4th larval instars *S. littoralis* at different constant temperature

Temp. °C	Alpha esterase (EST- α) Ug α -naphthol/min/g.b.wt Mean \pm S.E	Beta esterase (EST- β) Ug β -naphthol/min/g.b.wt Mean \pm S.E	Ache Ug Ach Br/min/g.b.wt Mean \pm S.E
15	3036.7 \pm 81.19 ^b	3752.0 \pm 141.64 ^b	47.3 \pm 1.45 ^{bc}
20	6566.7 \pm 114.44 ^b	3603.6 \pm 116.71 ^a	76.0 \pm 2.51 ^b
25	6985.7 \pm 86.61 ^a	4774.3 \pm 94.12 ^{ab}	242.3 \pm 13.01 ^a
30	4509.7 \pm 110.87 ^c	4319.3 \pm 602.31 ^{ab}	216.0 \pm 11.37 ^a
35	2987.3 \pm 36.67 ^d	1726.7 \pm 109.54 ^c	33.7 \pm 2.72 ^c

Note: Means with the same letter in the same row are not significantly different

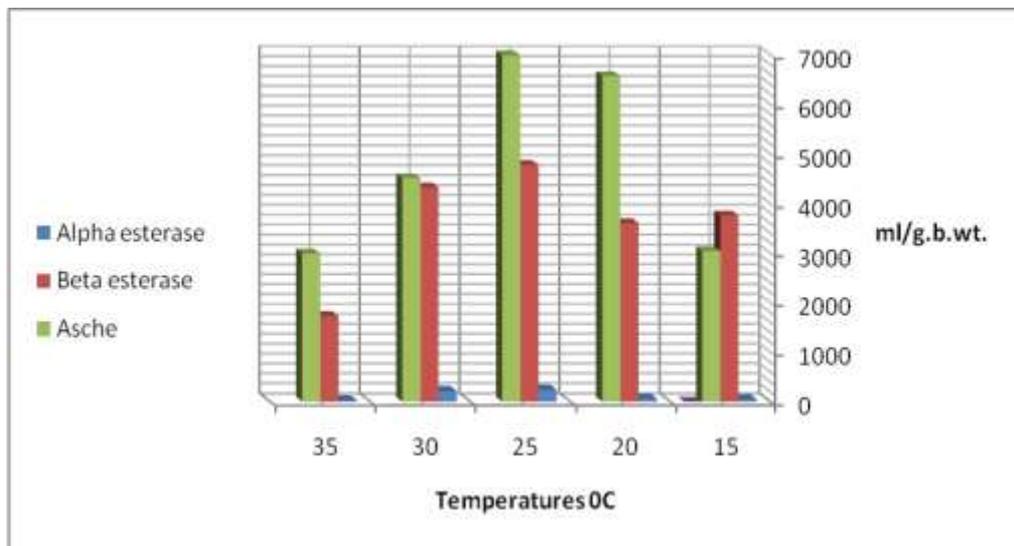


Fig (4): The activity levels of acetylcholinesterase (AChE), Alpha esterase (EST- α), and Beta esterase (EST- β) of 4th larval instars *S. littoralis* at different constant temperatures.

Our results disagree with Debora *et al.*, (2016) who concluded that AChE activity decreased at higher temperatures and corroborating the results of Domingues *et al.*, (2007) who observed that the activity of the AChE of *Chironomus riparius* Meigen, is higher at 6 °C and 16 °C than at 26 °C. These results also disagree with finding of Débora *et al.*,(2016) who studied effects of different temperature on *Chironomus sancticaroli* where he found AChE activity decreased with increasing temperatures: at 20 and 25 °C, it was 69% and 59% lower than at 30 °C, respectively. No significant changes in enzyme activity were detected between 20 and 25 °C. No changes in the activity of EST- α were observed between 20 and 25 °C. However, at 30 °C the enzyme activity increased by 44% and 45% when compared to 20 and 25 °C, respectively. The enzyme activity of EST- β was high at the intermediate temperature of 25 °C. At this temperature, EST- β activity was 24% higher than at 20 °C and 18% higher than at 30 °C. In contrast, enzyme activity at 20 and 30 °C did not differ. Singh *et al.*, (2013) concluded that *Pricini* when exposed to low temperature cause alterations in the activity and kinetics of the tissue. Thus AChE activity appears to be a potential biomarker towards the evaluation of the impact of cold stress on silk worms.

REFERENCES

- Ali, A.; Rashid, M.A.;Huang, Q.Y.; Wong, C. andLei, C.L. (2017). Response of antioxidan enzymes in *Mythimna separata* (Lepidoptera: Noctuidae) exposed to thermal stress.Bull Entomol Res. Jun; 107(3):382-390.
- AN, M. I. and Choi, C. Y.(2010).Activity of antioxidant enzymes and osmotic physiological responses in ark shell, *Scapharca broughtonii*, exposed tothermaland stress: effects on hemolymph and biochemical parameters.Comp.Biochem. Physiol. Part B 155: 34-42.
- Angilletta, M. J. (2009). Thermal adaptation: a Theoretical and empirical synthesis. Oxford University Press, Oxford.
- Asperen, V. K. (1962): A study of housefly esterase by means of sensitive colorimetric method. *J. Insect Physiol.*, 8: 401-416.
- Bale, J.S.; Masters, G.J.; Hodkinson, I.D. ;Awmack, C.;Bezemer, T.M.; Brown, V.K.;Butter field,J.Buse,A.;Coulson, J.C. and Farrar, J. (2002).Herbivore in global Climate change research: direct effects of rising temperature on insect herbivores .Glob Change Biol .8: 1–16.
- Berggren, A.; Bjorknam C.;Bylund, H.and Ayres M.p.(2009).The distribution and abundance of animal populations aclimate of uncertainty. OIKOS,118(8),1121-1126.
- Bradford, M.M. (1976).A rapid and sensitive method for the quantization of *Microgram quantities* of protein utilizing the principle of protein- dye binding Anal. Biochem.,72;248-254.
- Brooker,R.J.(2011).Genetic analysis and principle. New York, Ny; McGrow-hill Science.
- Callaghan, A.; Fisher T.C.; Grosso, A.; Holloway, G.J. andCrane,M. (2002). Effectof temperature and pirimiphosmethyl on biochemical biomarkers in *Chironomus riparius* Meigen. Ecotoxicol. Environ. Saf., 52, pp. 128-133.
- Coracini,M.D.A.;Zarbin,P.G.; Bengtsson, M.A. ; kovalski,A.; Vilela E.F.; Torezen,L.L;Hickel, E.R. and Witzgall,P.(2007). Effect of photoperiod and temperature on the development of *Bonagotacr anaodes*. Physiol. Entomol. 32:394-398.

- Costamagna, A.C. and Landis, D.A. (2004). Effects of food resources on adult *Glyptapanteles militaris* and *Meteorus communis* (Hymenoptera: Braconidae), parasitoids of *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). *Environ. Entomol.*, 33: 128-137.
- Cui, X. H.; Wan, F. H.; Xie, M. and Liu, T. X. (2008). Effects of heat shock on survival and reproduction of two whitefly species, *Trialeurodes vaporariorum* and *Bemisia tabaci* Biotype B. *J. Insect. Sci.* 8, 1–10.
- Dahi, H. F.; El-Sayed, Y.; El-Barkey, N. and Abd-El Aziz, M. (2009). Toxicological and biochemical studies of methylamine avermectin, a new type of bioinsecticide against the cotton leafworm, *Spodoptera littoralis*. *Egypt. Acad. J. Biol. Sci. (A. Entomology)* 2: 103-116.
- Débora, R. B.; Vinicius, R. S.; Maiara, V.; Guiloski, H. C.; Silvade Assis and Mário, A. N. S. (2016). Factors that alter the biochemical biomarkers of environmental contamination in *Chironomus ancticaroli* (Diptera, Chironomidae). *Revista Brasileira de Entomologia* Volume 60, Issue 4, Pages 341-34
- Denlinger, D. L. and Yocun, G. D. (1998). Physiology sensitivity in insects and application in integrated pest management (eds. G. J. Hallman & D. L. Denlinger), pp. 6–53. Westview Press, Colorado.
- Diffenbaugh, N. S. (2005). Fine-scale processes regulate the response of extreme events to global climate change, *Proc. Natl. Acad. Sci. U.S. A.*, 102, 15, 774–15, 778.
- Domingues, I.; Guilhermino, L.; Soares, A. M. V. M., and Nogueira, A. J. A. (2007). Assessing Dimethoate contamination in temperate and tropical climates: Potential use of biomarkers in bioassays with two chironomid species. *Chemosphere*, 69(1), 145-154.
- Dooremalen, A. C. V.; Koekkoek, B. J. and Ellers, A. J. (2011): Temperature-induced plasticity in membrane and storage lipid composition: Thermal reaction norms across five different temperatures. *Journal of Insect Physiology* 57: 285–291.
- Easterling, D. R.; Meehl, G. A.; Parmesan, C.; Changnon, S. A.; Karl, T. R. and Mearns, L. O. (2000). Climate extremes: observation modeling, and impacts *Science*, 389: 2068-2074.
- El-Aswad, A. F.; Abdelgaleil, S. A. M. and Nakatani, M. (2003). Feeding deterrent and growth inhibitory properties of limonoids from *Khaya senegalensis* against the cotton leafworm, *Spodoptera littoralis*. *Pest Manag. Sci.* 60, a199-203.
- Ehsan, B. M.; Izadi, H. Samih, A. M.; Moharramipour and Mahdian, K. (2011). Effect of Insect Growth Regulators, Temperature and Overwintering on Larvae of Pistachio Leaf White Borer, (*Oncideria terebinthina*). *Int. J. Agric. Biol.*, Vol. 13, No. 3, 2011
- Ellis, J. r. j. D.; Neumann, R. H. and Elaz, P. J. (2002). Longevity and Success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural reproductive diets. *J. Econ. Entomol.*, 95: 902-907.
- Englund, G.; Ohlund, G.; Hein, C. L. and Diel, S. (2011). Temperature dependence of the functional response. *Ecology letters* 14, 914-921.
- Gamil, W. E. (2004). Production of some bio-formulations and study of their efficiency on some physiological traits in some insect species. Pp 32. M. Sc. Fac. of Agric. Ein Shams Univ., Egypt. 135pp.
- Gullan, P. J. and Cranston P. S. (2005). The insects: an outline of entomology. 3rd ed. Black well Publishing Ltd., Davis, USA.
- Habig, W. H.; Pabst, M. J. and W. B. Jakoby (1974). Glutathione S-transferase the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249

- (22): 7130-7139.
- Hachiya, T.; Terashima, I. and Noguchi, K. (2007) Increase in respiratory cost at high temperature is attributed to high protein turnover cost in *Petunia* x hybrid petals. *Plant, Cell & Environment*, 30, 1269–1283.
- Hag, C.; Steel, D.; Marchand I.; Seiber, C. and Brody, D. (2004). Integrating the art and Science of medical practice innovations in teaching medical communications skill family medicine, 36(1; suppl), pp.43-50.
- Hansen, J. D.; Johnson, J. A. and Winter, D. A. (2011). History and use of heat pest control: a review. *Int. J. Pest Manage.* 57, 267–289.
- Hochachka, P.W. and Somero, G.N. (1973). Strategies of biochemical adaptation. WB Saunders, Philadelphia, 16.
- Hochachka, P. W. and Somero, G. N. (2002). Biochemical adaptation: mechanism and process in physiological evolution. New York: Oxford University Press.
- Ishaaya, I.; Moore I. and Joseph, D. (1971). Protease and amylase activity in larvae of the Egyptian cotton leafworm, *Spodoptera littoralis*. *J. Insect Physiol.*, 17: 945-953.
- Ishaaya, I. and Swirski E. (1976). Trehalase, invertase and amylase activities in the black scale, *Saissetia oleae*, and their relation to host adaptability. *J. Insect Physiol.* 22: 1025-1029.
- Kar, P.K.; Srivasta, P.P.; Sinha R.K. and Sinha, B.R.R.P. (1994). Protein concentration in the pupal haemolymph of different races and F1 stop cross of *Antheraea mylitta* D. *Indian. J. Seric* 33(2):174-175.
- Khrüt, U.; Samietz J. and Dorn S., (2006). Thermal response in adult codling moth. *Physiol. Entomol.*, 31: 80-88. |
- Kingsolver, J.G. (2006). Thermal reaction worm for caterpillar growth depend on diet. *Evolutionary Ecology Research*. 8:703-715.
- Kiran, k., N.; Ismail S.M. and Dutta, A. (1998). Differential uptake of storage protein by the fat body of Rice moth, *Corcyra cephalonica* during the larval pupal Develop. *Entomon*, 23: 83-90.
- Knight, J. A.; Anderson S. and Rawle J. M. (1972): Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipids. *Clin. Chem.*, 18: 199-202.
- Kumar, K.; Gupta, C.S.; Baidoo, K.S.; Chnder, Y. and Rosen, J.C. (2005). Antibiotic Uptake by Plants from Soil Fertilized with Animal Manure *Journal of Environmental Quality* Vol. 34 No. 6, p. 2082-2085
- Lale, N.E.S. & Vidal, S. (2003). Effect of constant temperature and humidity On oviposition and *Callosobruch hussubinnotatus* (pic) on bambara Ground nut, *Vigna subterranean* (L.) *verdcourt*. *J. stored. Prod. Res.*, 39:459-470 .
- Laufer, H. Knight, J. A.; Anderson S. and Rawle J. M. (1972): Chemical basis of the sulfophospho-vanillin reaction for estimating total serum lipids. *Clin. Chem.*, 18: 199-202.
- Lee, H., Stultz, B.G., Hursh, D.A. (2007). The Zic family member, odd-paired, regulates the *Drosophila* BMP, decapenta plegic, during adult head development. *Development* 134(7): 1301--1310.
- Lee, K.P. and Roh, C. (2010). Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. *Entomologia Experimentalis et Applicata*, 136, 151–163.
- Lee, K.P.; Behmer, S.T. and Simpson, S.J. (2002). A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval).

- Journal of insect Physiology, 48,655-665.
- Lee, K.P.; Raubenheimer, D. and Simpson, S.J. (2004). The effects of nutritional imbalance on compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar. *Physiological Entomology*, 29, 108–117.
- Lee, Y. P. and Takabashi, T. (1966). An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.* 14: 71-77.
- Lemoine N. P., and Burkepile D. E. (2012). Temperature-induced mismatches between consumption and metabolism reduce consumer fitness. *Ecology*, 93, 2483–2489.
- Malik, M.A and Malik, F. A. (2009). Oncogenic changes in haemolymph biochemical composition in the Silkworm, *Bombyx mori* L under Thermal Stress *Academic Journal of Entomology* 2 (1): 16-21.
- Martin, M.D. (1969). Developmental changes in the late larva of *Calliphora stygia*-II. Protein synthesis *Aust. J. Biol. Sci.*, 22: 935-945.
- Mukanganyama, Z. B.; Fathipour, Y.; Khanjani, M.; Moharramipour S. and Zaluki, M. (2010). Preimaginal development response to constant temperatures in *Hypera postica* (Coleoptera: Curculionidae): picking the best model. *Environ. Entomol.* 39: 177–189.
- Nagata, M. and Kobayashi, M. (1990). Effects of nutrition on storage protein concentration in the larval haemolymph of silkworm, *Bombyx mori* L. *J Sericul. Sci. Japan*, 59: 469-474.
- Naumff, D.G. (2001). β -Fructosidase superfamily: homology with some α -L-arabinases and β -D xylosidases. *Prot. Struc. Functions and Gen.* 42:66-76
- Neven, L.G. (2000). *Physiological responses of insects to heat*. *Postharvest Biology and Technology*. 21:103-111.
- Parmesan, C., and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421: 37–42.
- Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematic* 37, 637 - 669.
- Pant ,R.; and Gupta ,D. (1979). The effect of exposure to low temperature on the metabolism of carbohydrates, lipids and protein in the larvae of *Philosamia ricini* R J. *Biosci.*, Vol. 1, Number 4, December 1979, pp. 441–446.
- Pineda, S.; Budia, F.; Schneider, M. I.; Gobbi, A.; Vinuela, E.; Valle J. and Del Estal, P. (2004). Effect of two biorational insecticides, Spinosad and methoxy fenoxycarb, on *Spodoptera littoralis* (Lepidoptera: Noctuidae) under laboratory conditions. *J. Econ. Entomol.*, 97 (6): 1906-1911.
- Rinehart, N.J.; Bradshaw, J.L.; Moss, S.A.; Brereton, A.V. and Tonge, B.J. (2000). Atypical interference of local detail on global processing in high-functioning autism and Asperger's disorder. *J Child Psychol Psychiatry.* ; 41(6):769-78.
- Schin, K.S. (1971): Quantitative studies of hydrolytic enzymes activity in the Salivary gland of *Chironomus tentans*. *Entomol.* 103:457.
- Simpson, D. R.; Bull, D. L. and Lindquist, D. A. (1964): A semi micro technique for the estimation of cholinesterase activity in bull weevil. *Ann. Ent. . Soc. Am.*, 57 (3):367- 377.

- Singh, N. B. and Sinha, R. N. (1977). Carbohydrates, lipids and protein in the developmental stages of *Sitophilus oryzae* and *Sitophilus grannarius* *Ann.Ent. Sos. Am.* 107-111.
- Singh, A.; Sunil, K.; Jaiswal, and Sharma, B. (2013). Effect of low temperature stress on acetylcholinesterase activity and its kinetics in 5th instar larvae of *Philosamia ricini*. *Journal of Biochemistry Research*. Vol. 1(2), pp.17-25.
- Sonmez, E.; and Gulel, A. (2008). Effect of different temperatures on the total Carbohydrates, lipids and proteins amount of the bean beetles, *Acanthoscelides obtectus* say (Coleoptera :Bruchidae). *Pakistan Journal of Biological Science* V.11,N:14,1803-1808.
- Srinivasan, S. ;Dorigi, K.M. and Tamkun, J.W. (2008). Drosophila Kismet regulate histone H3 lysine 27 methylation and early elongation by RNA polymerase II. *PLoS Genet.* 4(10): e1000217.
- Stone A.C. ,Gehring C.A.& Whitham (2010).Drought negatively affect communities on a foundation tree Growth ring predict diversity.*Oecologia*,164,751-761.
- Sultan, R.; Mohammad, M. and Ali, R. B.(2009): Biochemical characterization of digestive amylase of wheat bug, *Eurygaster maura* (Hemiptera: Cutelleridae). *African Journal of Biotechnology* Vol. 8 (15),pp. 3640-3648.
- Takaya, I.(2005).Intrinsic optimum temperature for development of insects and mites *.Environ. Entomol.*34(6)1377D1387.
- Tatchell, R. J.; Araman, S. F. and Boctor, F. N. (1972): .Biochemical and physiological studies of certain Ticks (Ixodoidea).*Z.Parsitenk* .Vol 39:345-350.
- Taveras,R.; Hilje, L. and Carballo,M.(2004).Developmen of *Hypsipyla grandella* (zeller) (Lepidoptera : Pyralidae) in response to constant temperatures.*Neotrop.Entomol.*,33:1-6.
- Terra,R.W. and Ferreira,C.(2012).Biochemistry and molecular biology. *Insect Molecular Biology and Biochemistry*,pp.365-418.
- Tsujita. T.;Ninomiya, H. and Okuda, H. (1989) P-nitrophenyl butyrate hydrolyzing activity of hormonesensitive lipase from bovine adipose tissue. *J Lipid Res.* 30: 997-1004,.
- Wilce,M.C. and Parker M.W.(1994).Structure and function of glutathione S. transferases.*Biochim BiophysActa*16; 1205(1):1-18.
- Willmer, C.W.; Stone,G. and Johnston,I. (2004).Insecticidal action. *Proc. 4 Int. Cong. Biochem., Environmental physiology of animals* (Oxford: 201-210. Blackwell Science)
- Zhang, Q.; Tu, Y.Q.; Liu, H.; Zhang, D.; Zhang H.; Zhang,W.J.and Qin Y.S.(2016) .Effects of temperature stress on antioxidant enzymes in *Hepialusxia ojinensis* (*Lepidoptera: Hepialidae*) larva. *Journal of Environmental Entomology* , Vol.38 No.1 pp.47-53 ref.19.

ARABIC SUMMARY

التأثيرات المباشرة للتغيرات في درجات الحرارة علي الكيمياء الحيوية والانزيمية لدودة ورق القطن

عايدة سعيد كامل¹ - ولاء جميل ابراهيم² - حسن فرج ضاحي²

1- قسم علم الحشرات - كلية العلوم - جامعة بنها - مصر

2- معهد بحوث وقاية النباتات - مركز البحوث الزراعية- الجيزة - مصر

من المهم فهم آثار تزايد درجات الحرارة الناتج عن الاحترار العالمي على دودة ورق القطن المصرية، والتي تهاجم مدي واسع من المحاصيل الزراعية الهامة في الشرق الأوسط والتي تعتبر ابضا واحدة من الآفات الاقتصادية الرئيسية للقطن في مصر. ولذلك، تمت الدراسة علي خمس من درجات الحرارة الثابتة المختلفة ١٥، ٢٠، ٢٥، ٣٠ و ٣٥ م° كتأثيرات حرارية علي القياسات البيوكيميائية والانزيمية للعمر اليرقي الرابع لدودة ورق القطن المصرية. أجريت التحاليل البيوكيميائية المختلفة لتحديد آثار درجات الحرارة الثابتة المختلفة سابقة الذكر على كمية المجموع الكلي للبروتينات والدهون والكربوهيدرات والأحماض الأمينية الحرة بالإضافة للأنزيمات الخاصه بالأبيض والهضم والعصبية والمزيله للسموم. ويتضح من النتائج أن المجموع الكلي للبروتينات والأحماض الأمينية الحرة بالإضافة الي مستويات نشاط البروتينيز قد ارتفعت بدرجة معنوية وذلك بارتفاع درجات الحرارة لتصل إلى قمة نشاطها عند ٣٠ م°، بينما انخفض المجموع الكلي للدهون والكربوهيدرات انخفاضاً معنوياً عند ارتفاع درجات الحرارة، وبالإضافة لذلك، قد انخفضت مستويات نشاط الأميليز والإنفرتيز والتريهاليز عند درجة حرارة ١٥ م°، بينما وصل أنزيمي الأميليز والإنفرتيز الي قمة نشاطهما عند درجة حرارة ٣٠ م°، وفي نفس الوقت وصل التريهاليز الي قمة نشاطه عند درجة ٢٠ م° وكان هناك انخفاض حاد في أنشطة إنزيمات الكربوهيدرات عند درجة حرارة ٣٥ م°، ومن ناحية أخرى، كان هناك زيادة معنوية في مستوي الدهون الكلية والليبيز عند درجتى الحرارة المنخفضتين ١٥ و ٢٠ م°، بينما لا توجد اي معنوية بين باقي درجات الحرارة الاخرى. ولوحظ وجود زيادة معنوية في مستوي نشاط كل من Got , GPT حيث وصلت هذه الزيادة قمة نشاطها عند ٢٠ م° في حين كان التغير في مستوي الـ Got , GPT معنوياً عند باقي درجات الحرارة تحت الدراسة. كان هناك زيادة معنوية في مستوي انزيم GST عند درجة حرارة ٣٠ م° بينما وصل مستوي نشاطه الي اقل مستوي له عند درجة حرارة ١٥ م°، وعلاوة على ذلك، كانت هناك زيادة تدريجية علي درجات حرارة ١٥ و ٢٠ م° في كمية الاستيل كولين استيريز AChE والفا وبيتا استيريزز Alpha (EST-α) and beta esterase(EST-β)، حيث أنها وصلت إلى قمة نشاطها عند درجة حرارة ٢٥ م° وتبع ذلك انخفاض في مستوي النشاط علي درجتى ٣٠ و ٣٥ م°.